PRENATAL SEX DETERMINATION
HOW, WHEN AND WHY?

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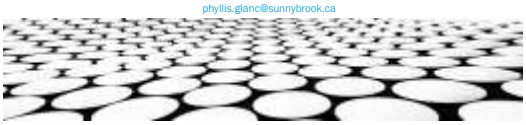

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OBJECTIVES: Prenatal Sex Determination

- Review Disclosure Guidelines, Indications
- Ultrasound fetal sex determination
  - External Genitalia
  - Internal Reproductive Organs
- Disorders of Sex Development (DSD)
  - Case Examples

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DETERMINATION & DISCLOSURE GUIDELINES
SOGC 2017 (Canada)

- Routine to evaluate external genitalia but not indication to prolong/repeat study
- Re-affirmed women have a right to their own PHI (Supreme Court 1992)
- Concerns risk termination if "not wanted fetal sex" are best addressed by the health professionals providing care for these women
- If unit wishes non-disclosure policy then information must be provided on report

https://doi.org/10.1016/j.jogc.2017.04.011

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DETERMINATION & DISCLOSURE GUIDELINES
2019 Denmark

- Minimal views should include direct visualization sagittal, transverse, tangential in T2
  - Sagittal only if < 15-16 wk.
- Fetal sex is disclosed on parental request
  - 95.8% had already discussed desire to know prior to onset of US (Swedish SWEPP study, Larsen et al)
  - 60-75% wish to know
- Overall accuracy sex determination 99.2% with incorrect determination 0.8%, primarily females
  - 8/1000 incorrectly assigned fetal sex

Romosan G, Henriksson E, Rylander A et al. Diagnostic performance of routine ultrasound screening for fetal abnormalities in an unselected Swedish population in 2000-2005. Ultrasound Obstet Gynecol 2009;34:526-33. Stærup, Gitte, et al. "Foetal sex determination at the second trimester anomaly scan in Denmark." *Danish Medical Journal* 66.12 (2019):DStærup, Gitte, et al. "Foetal sex determination at the second trimester anomaly scan in Denmark." *Danish Medical Journal* 66.12 (2019

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SPECIFIC INDICATIONS PRENATAL SEX DETERMINATION

1. Twins ≥ 14 weeks (55% DCDA twins are discordant for sex)
2. Recessive X-linked disease (affect male fetus)
3. US suspected anomalies including ambiguous genitalia, hypospadias.....
4. Genotype-phenotype discordance (discordant external genitalia sex determination and the genetic sex (karyotype)
   - Consider placental mosaicism, demise/unknown twin, maternal malignancy, transplant organ from male donor
   - Assessment internal reproductive organs may be helpful

- Swedish study midtrimester demonstrates genital malformation 3.8/1000 (minor); 0.7/1000 (major)
- > 70 genetic or syndromic associations

BJOG 2018 NIPT for fetal sex determination: is ultrasound still relevant? *Colamaria et al* ***2018***
Diagnostic Approach in prenatally detected genital abnormalities. D. Chitayat & P Glanc UOG 2010;35;637-646Romosan G, Henriksson E, Rylander A et al. Diagnostic performance of routine ultrasound screening for fetal abnormalities in an unselected Swedish population in 2000-2005. Ultrasound Obstet Gynecol 2009;34:526-33;

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cfDNA and Fetal Sex Determinaton

- Sensitivity/specificity NIPT for fetal sex determination is 0.989/0.996, respectively*
  - Accurate very early in pregnancy from 7 weeks
  - US based sex determination accuracy approaches NIPT ~ 14 week GA
- Sex Chromosome Aneuploidy (SCA) include monosomy X, XXX, XXY, XYY
  - Sex chromosome mosaicism occur up to 50% thus SCA FPR 0.4%, PPV range 25-52%

*Mackie FL, Hemming K, Allen S, Morris RK, Kilby MD. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *BJOG*. 2017;**124**(1):32-46.Sofer, Laurel, et al. "Impact of cell-free DNA screening on parental knowledge of fetal sex and disorders of sex development." *Prenatal Diagnosis* 40.11 (2020): 1489-1496. Bowman-Smart, Hilary, et al. "Sex selection and non-invasive prenatal testing: A review of current practices, evidence, and ethical issues." *Prenatal diagnosis* 40.4 (2020): 398-407.

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PRENATAL SEX DETERMINATION ACCURACY BY ULTRASOUND

- First Trimester : 70% (11wk); 97% (12wk); 100% (13 wk)
- 2nd/3rd Trimester: Accuracy assignment range 87-100% male versus 92-100% female
  - Failure to assign sex 9-35%

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WHY IMPORTANT: PRENATAL DIAGNOSIS OF SEX DISCORDANCE

- Increasingly common prenatal detection DSD due
  - Desire to know fetal sex
  - Early Integration cfDNA into practice provide sex chromosome assessment in many < 10 weeks, thus discordant fetal sex more commonly diagnosed
    - 1/1500-2000 pregnancies.
- Early detection permits appropriate investigations and timely intervention as applicable
- Diagnosis of DSD may be source extreme parental anxiety

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SEXUAL DIFFERENTIATION IS COMPLEX PROCESS

- First 6 weeks bipotential state (hormone independent)
- Subsequent development external genitalia into characteristic male complete by 12 weeks in male, later in female

Diagnostic Approach in prenatally detected genital abnormalities. D. Ohlsyat & P Glanc, UOG 2010;35:637-646

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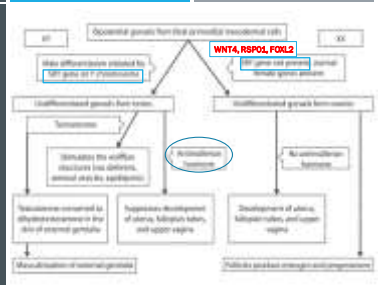
SEXUAL DIFFERENTIATION IS COMPLEX PROCESS

- Genetic control of differentiation involves the complex interaction of SRY, WT1, SF1, SOX9, fibroblast growth factor 9, PGD2, and DAX1 among others
- XX is not pure default status but requires expression of genes (WNT4, RSPO1, FOXL2) for normal female differentiation



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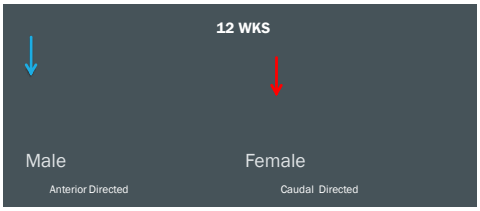
MALE OR FEMALE EXTERNAL GENITALIA PHENOTYPE SAGITTAL SIGN



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Male or Female External Genitalia Phenotype Sagittal Sign

9 WEEKS

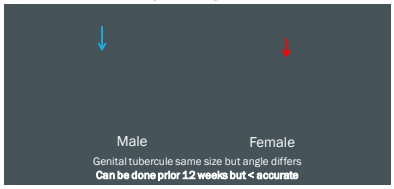

Figure 1in Chitty, Lyn S., et al. "Prenatal management of disorders of sex development." *Journal of pediatric urology* 8.6 (2012): 576-584.

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MALE OR FEMALE EXTERNAL GENITALIA PHENOTYPE SAGITTAL SIGN

≥ 12 wks > 95% accurate

- Draw lines along genital tubercle & horizontal line LS skin midsagittal plane
- Male angle > 30 degrees, anterior direct genital tubercule (angle increase with advancing GA)
- Female angle < 10 degrees, caudally direct genital tubercule
- Indeterminate between 10-30 degrees

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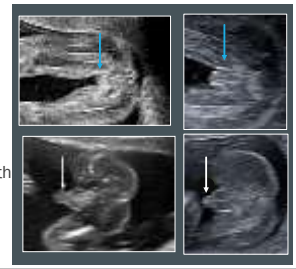
PRENATAL SEX DETERMINATION ROUTINE MID SECOND TRIMESTER

- Female
  - 3 parallel lines of labia majora & apposed labia minora
- Male
  - Septate dome shape scrotum with nonseptate penis arising from base



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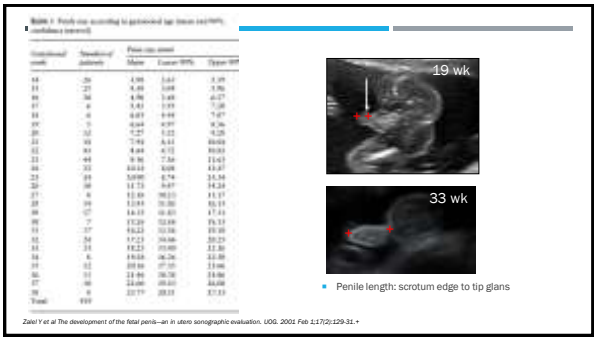
19 wk
33 wk
Penile length: scrotum edge to tip glans
Zalel Y et al The development of the fetal penis—an in utero sonographic evaluation. UOG. 2001 Feb 1;17(2):129-31.+

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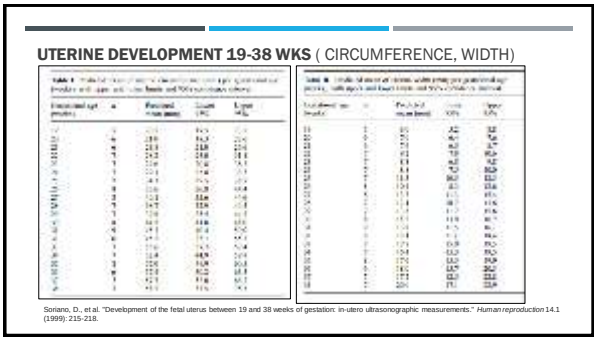
UTERINE DEVELOPMENT 19-38 WKS (CIRCUMFERENCE, WIDTH)
Soriano, D., et al. "Development of the fetal uterus between 19 and 38 weeks of gestation: in-utero ultrasonographic measurements." Human reproduction 14.1 (1999): 215-218.

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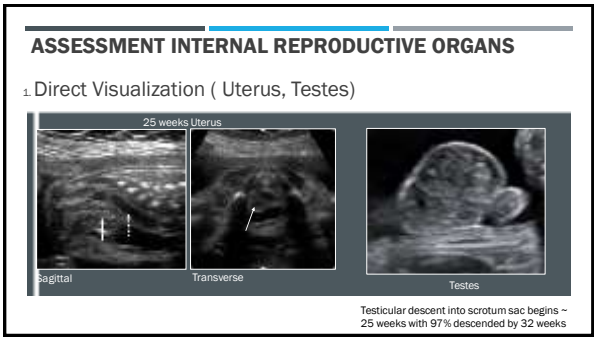
ASSESSMENT INTERNAL REPRODUCTIVE ORGANS
1. Direct Visualization (Uterus, Testes)
25 weeks Uterus
Sagittal
Transverse
Testes
Testicular descent into scrotum sac begins ~ 25 weeks with 97% descended by 32 weeks

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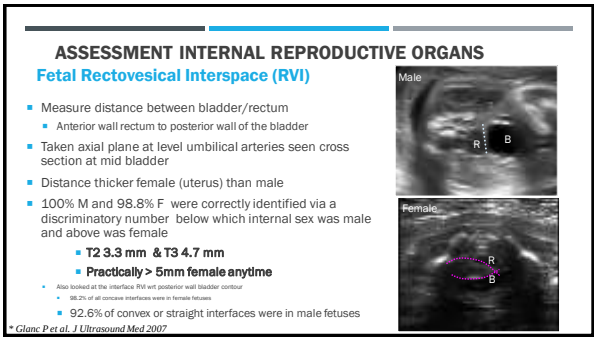
ASSESSMENT INTERNAL REPRODUCTIVE ORGANS
Fetal Rectovesical Interspace (RVI)
Measure distance between bladder/rectum
Anterior wall rectum to posterior wall of the bladder
Taken axial plane at level umbilical arteries seen cross section at mid bladder
Distance thicker female (uterus) than male
100% M and 98.8% F were correctly identified via a discriminatory number below which internal sex was male and above was female
T2 3.3 mm & T3 4.7 mm
Practically > 5mm female anytime
Also looked at the interface RVI wrt posterior wall bladder contour
98.2% of all concave interfaces were in female fetuses
92.6% of convex or straight interfaces were in male fetuses
* Glanc P et al. J Ultrasound Med 2007
Male
R
B
Female
R
B

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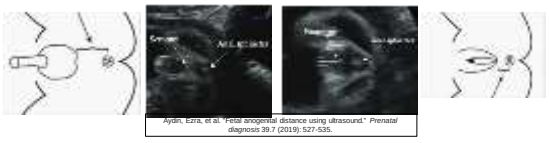

Aydin, Ezra, et al. "Fetal anogenital distance using ultrasound." *Prenatal diagnosis* 39.7 (2019): 527-535.

ANO-GENITAL DISTANCE (AGD)

- AGD is a sexually dimorphic measure, androgen is driver of AGD length, reliable after 21 weeks but can be done earlier
  - 11-13 wks: ≥4.8 mm males; < 4.8 mm females
- Measure from center anus to posterior convergence fourchette (female) or to base scrotum (male)

Sipahi, Mehmet, Vehbi Yavuz Tolgbz, and Şebnem Alanya Tosun. "An appropriate way to predict fetal gender at first trimester: anogenital distance." *The Journal of Maternal-Fetal & Neonatal Medicine* 32.12 (2019): 2012-2016. Aydin, Ezra, et al. "Fetal anogenital distance using ultrasound." *Prenatal diagnosis* 39.7 (2019): 527-535.

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DISORDERS OF SEX DEVELOPMENT (DSD)

- Atypical chromosomal, gonadal and/or anatomic sex development
- Present discordant genetic sex and ultrasound phenotype external genitalia
  - Commonest presentation at onset puberty
  - 1-2% incidence
  - Require multidisciplinary approach

Chitayat, D., and P. Glanc. "Diagnostic approach in prenatally detected genital abnormalities." *Ultrasound in Obstetrics & Gynecology* 35.6 (2010): 637-646

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Sex Chromosome DSD	46,XY, DSD	46, XX, DSD
45, X	Complete gonadal dysgenesis	Disorder Gonadal Development
47, XXY	Partial gonadal dysgenesis	• Ovotesticular DSD
45, X/46, XY	Gonadal regression	• Testicular DSD (SRY+,DupSOX9)
46, XX/46, XY	Ovotesticular DSD	• Gonadal dysgenesis
	Disorder in Androgen Synthesis or Action	**Androgen Excess**
	• Androgen biosynthesis defect (17-hydroxysteroid dehydrogenase, 5-reductase)	• Fetal (21- or 11-hydroxylase deficiency)
	• Defect in androgen action (AIS)	• Feto-placental (aromatase deficiency)
	• LH receptor defects	• Maternal
	• Disorders of AMH and AMH receptor (persistent Mullerian duct syndrome)	
	Others (severe hypospadias, cloacal exstrophy, OEIS,....)	

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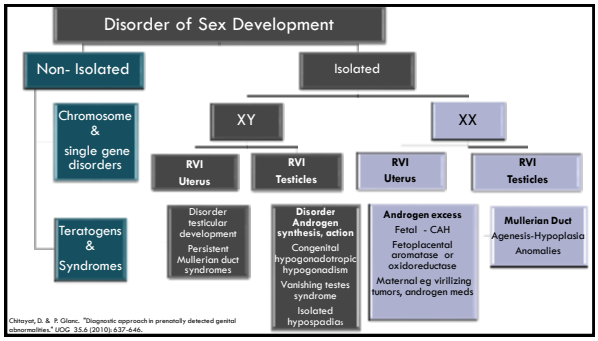

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Step			
1	**Describe external genitalia**	Direct visualization descended testes or uterus	Determine RVI or rectovesical interspace diameter
2	**? Isolated**	Isolated commonest	Non-isolated include Trisomy 13,18,triploidy, deletions(4,11,13), syndromes, associations, IUGR, teratogens • > 70 syndromes/associations :Smith Lemli Opitz, Velocardiofacial S, Prader-Willi S, VACTERL, CHARGE, Fraser S, OEIS....
3	**Pregnancy history**	**Maternal virilization due Exposure** Androgens, endocrine disruptors **(phenytoin, aminoglutethimide), teratogens (pesticides, tretretinoin), alcohol, progestin, danazol, cimetidine, spironolactone)**	**Maternal Serum Biochemistry** UPI (High AFP & bHCG, low PAPP-A); Low Estriol (Smith-Lemli Optiz, CAH, placental aromatase deficiency, T18), **maternal androgan producing tumors, CAH**
4	**Family history**	Consanguinity & AR CAH, androgen biosynthesis, Fraser S Infertility or amenorrhea may indicate AIS	Unexplained SIDS ?Salt loosing CAH Other recurrent miscarriages, stillbirths, inherited conditions....
5	**Lab**	**Mutation analysis** 21 hydroxylase deficiency, 5α reductase deficiency, SRY mutation	**Check amniotic fluid for additional hormones** 17-hydroxy-progesterone, testosterone, androstenedione, 11-dexoycortisol and 7-dehydrocholesterol
6	**Differential**	Isolated Ammiocentesis chromosome, amniotic fluid hormones	Non-isolated consider teratogens, chromosome/gene disorders, associations, syndromes

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CASE #1

AMBIGUOUS GENITALIA

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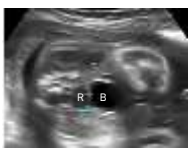
CASE #1

46, XY, DSD

Findings US: "tulip sign" of marked hypospadias



Incomplete Masculinization External Genitalia

- Abnormal production or conversion testosterone
- Abnormal response to normal levels of testosterone
  - AIS (androgen insensitivity syndrome)
  - May present with inguinal swelling neonate or amenorrhea puberty

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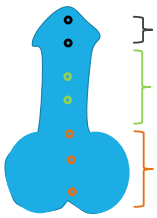

1st degree open below tip, glandular (50%)

2nd degree urethral opens along penile shaft (30%)

3rd degree (20%) urethral opens penoscroal junction, scrotal or below penis (perineal)

INCOMPLETE MASCULINIZATION EXTERNAL GENITALIA

HYPOSPADIAS

- Range 1/250- 2-4/10,000
- Look abnormally positioned ventral jet of urine
- Increased prevalence background maternal meds such as steroids, progestin, valproic acid
- Increased prevalence early onset severe IUGR

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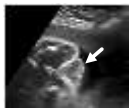
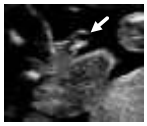
HYPOSPADIAS

- "Tulip" sign present in more severe cases hypospadias where we note the blunted bulbous tip of the penis and parallel echogenic lines of the lateral folds of the foreskin/scrotal folds
- Penile shaft foreshortened or buried between scrotal folds, may curved (chordee)
- May associated meatal stenosis with urethral dilation and high micturition velocities





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CASE # 2

27 WEEKS

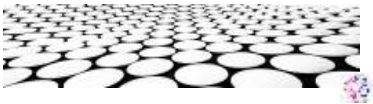
AMBIGUOUS GENITALIA

HYPOSPADIAS

fetalmedicine.org/var/uploads/VideoArticle/495/genital_tract_ambiguous_genitalia

Micturition occurring along shaft penis

Micturition tip penis Normal



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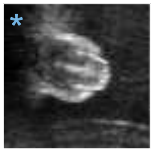
CASE # 4

Ambiguous Genitalia

46, XX, DSD



* (Image :Courtesy T.E. Cohen-Overbeek and I.A.L. Groenenberg, Department of Obstetrics and Gynaecology, Erasmus MC, Rotterdam). Figure 3 in : Chitty, Lyn S., et al. "Prenatal management of disorders of sex development." *Journal of pediatric urology* 8.6 (2012): 576-584.

VIRILIZATION EXTERNAL GENITALIA

- Commonest etiology is CAH
  - Look at adrenals
- Clitoromegaly without enlargement labial folds
- Look maternal virilization, consider inherited, exogenous drugs, teratogens, maternal tumors.....

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CASE #4: 46,XX, DSD Congenital Adrenal Hyperplasia

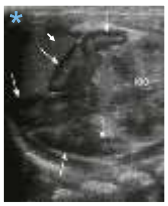
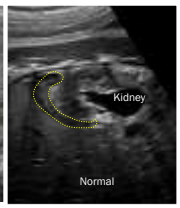


*Image courtesy:Bronshtein et.al. 1993



Term length < 20mm, limb diameter < 4mm
Smooth

- Commonest etiology 46,XX,DSD phenotype discordance with female virilization
- 1/14,000 (virilization XX)
- AR (25% risk recurrence)
- 90% enzyme deficiency 21-hydroxylase deficiency (USA neonatal screen)
- 75% combined with salt loosing form associated risk early neonatal death
- If concern may initiate treatment low dose steroid to minimize female virilization by 4 weeks

Tardy-Guidollet et al."New management strategy of pregnancies at risk of congenital adrenal hyperplasia using fetal sex determination in maternal serum. *J Clinical Endocrinology & Metabolism* 99.4 (2014):1180

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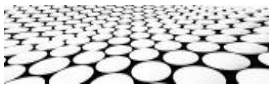
CASE #5

29 yo, transferred 30.5 wk GA

- Maternal hypertension, elevated LFTs
- Fetal oligohydramnios, absent EDF in UA, FGR 5th percentile



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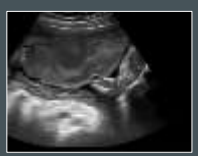
XY DSD
Severe hypospadias
Placental dysfunction with IUGR



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RELATIONSHIP IUGR & HYPOSPADIAS

- Hypospadias in 0.3-0.4% general population
  - Most isolated, believed multifactorial
  - Incomplete fusion of urethral folds between 7th and 14th GA
- Most severe form of placenta mediated IUGR occurs early in T1 (wks 7-8) when male genitalia forming
  - 1/3 hypospadias associated with uteroplacental insufficiency
  - * 19% incidence hypospadias/ambiguous genitalia in FGR
  - ** 60% incidence severe hypospadias in FGR, 92% also associated bidfid scrotum/micropenis/crytporchid etc.
  - ***FGR may also be associated with penile shortening and undescended testicles without hypospadias
  - ****Suggest placental hCG is critical for penile, urethral, testicular development

*Yinon, J Kingdom et al 2009 AJMG; ** Hashimoto et al. "Fetal growth restriction but not preterm birth is a risk factor for severe hypospadias." Pediatrics international (2016). ***Nimkc, Stefan F., et al. "Male genital abnormalities in intrauterine growth restriction." *Perinatal diagnosis* 32.5 (2012): 427-431.**** Ghazarian, Armen A., et al. "Placental weight and risk of cryptorchidism and hypospadias in the collaborative perinatal project." *American journal of epidemiology* 187.7 (2018): 1354-1361.

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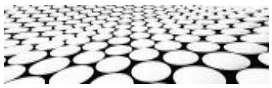
CASE # 6

- Ambiguous Genitalia at midtrimester study



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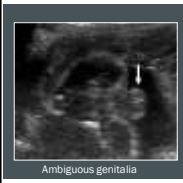
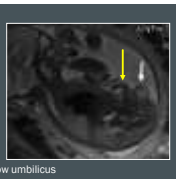
46, XY, DSD EPISPADIAS-EXSTROPHY COMPLEX



Ambiguous genitalia



mass below umbilicus

- No phallus identified US or MRI
- No bladder (everted represented by mass below umbilicus)

Images courtesy : Chitayat & Glanc. "Diagnostic approach in prenatally detected genital abnormalities." UOG 35.6 (2010): 637-646.

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EXSTROPHY-EPISPADIAS COMPLEX (EEC)

- Epispadias (dorsal) rarely isolated
- **Bladder exstrophy** always has epispadias (rarely isolated)
  - Non-visualized bladder & infraumbilical abdominal wall mass, abnormal genitalia
  - Dorsal opening urethral, dorsal/upward
  - 1 in 120,000 male vs 1 in 500,000 female births
  - Birth prevalence 1/30,000 bladder exstrophy; 1/200,000 cloacal exstrophy and OEIS
  - Severity related timing disruption cloacal membrane
- **Cloacal exstrophy** = + anal atresia plus 50% spinal/vertebral
- **OEIS** (omphalocele- exstrophy-imperforate anus-spinal defects) most severe form EEC

Epispadias (dorsal)

Hypospadias (ventral)

https://fetalmedicine.org/var/uploads/Video Article/279/abd_wall_cloacal_ext.mp4

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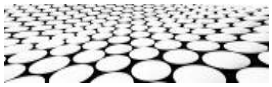
CASE # 7

- 21 weeks normal anatomy
- 27 weeks 2cm omphalocele
- 33 weeks referred to distinguish omphalocele vs gastroschisis



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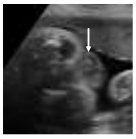
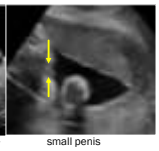


Testicles descended (XY,DSD)

Low abdominal wall defect (below umbilicus), no bladder



small penis

DIAGNOSIS: Bladder Exstrophy (based on absent bladder and infraumbilical abdominal wall defect)

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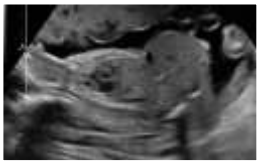
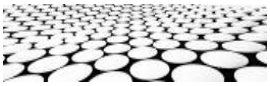
CASE # 7

34-year-old G2P1

Large abdominal wall defect





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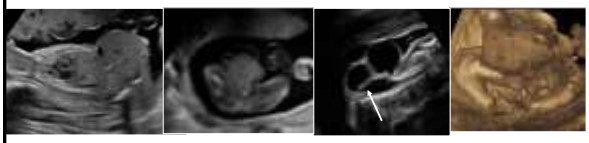
OEIS (*omphalocele, extrophy bladder-imperforate anus-spinal anomalies*



- Findings include omphalocele (contained SI, part liver, right testes) with defect below umbilicus
- Absent large bowel with persistent cloacal structure replacing bladder & rectum with ureters draining into it
- Lumbar myelomeningocele, clubfeet, single UA
- Failure fusion pubic rami & genital tubercles

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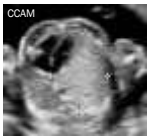
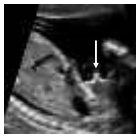
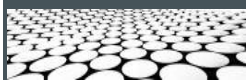
CASE # 8

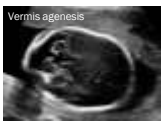
Routine midtrimester study

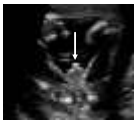
22 weeks











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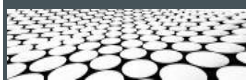
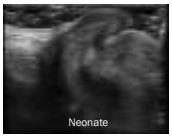
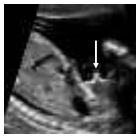
CASE # 8 46 XY DSD

Multiple anomalies including micropenis

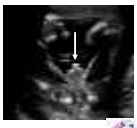
- Defect hypothalamic-pituitary-gonadal axis
- Associated cryptorchid, small testicles, ICSI
- Treatment testosterone







Micropenis in neonate = SPL (stretched penile length < 1.9 cm)



46,XY DSD with isolated micropenis are raised as males, e no surgical intervention, potential for fertility
- gender satisfaction equal to patients with the same disorder who are raised as females

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SEX ASSIGNMENT

Evolving

Multidisciplinary approach

- Consider: diagnosis, karyotype, appearance external genitalia, type of gonads present, ? surgical interventions, sex of rearing, hormonal therapy, potential for fertility, parental wishes & cultural beliefs **and Gender Identity of the Individual**.
- **Knowledge of etiology may aid in determining sex assignment while consider gender identity individual**
  - 46,XX DSD genotype and CAH (majority raised female, fertility potential)
  - 46,XY DSD genotype with complete gonadal dysgenesis or 46,XY genotype and complete androgen insensitivity syndrome (AIS); thus external genitalia appear female phenotype
  - 46,XY DSD with micropenis gen raised males, no surgical intervention; potential for fertility, gender satisfaction ~ same if raised as females
  - 46XY DSD 5a-reductase deficiency, variable degree under masculinization
    - Mendonca et al reported 50% with a 5a-reductase deficiency raised female later self-identified as males
  - Gonadal dysgenesis may have uterus which can impregnate with donor oocytes

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CONCLUSION: PRENATAL SEX DETERMINATION

COMPLEX EVOLVING AREA

- Prenatal sex determination routine, disclosure variable
- External genitalia for sex determination highly reliable > 14 weeks
- Setting of discordant chromosome and phenotype external genitalia options:
  - Direct visualization uterus/testes and/or measure RVI
  - Check adrenals and placenta function indices, determine if isolated, review relevant maternal/FH , drug history, labs, mutation analysis
- DSD challenge for family and healthcare team, final decision may wait till puberty

Chitayat, D., and P. Glanc. "Diagnostic approach in prenatally detected genital abnormalities." UOG 35.6 (2010): 637-646.

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THANK YOU

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